

Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein

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Despite differences in initiating events and pathophysiology, the aetiological agents of all autoimmune diseases are lymphocytes specifically reactive against normal constituents of the individual. Recently we have isolated and grown as a cell line rat T lymphocytes reactive against myelin basic protein (BP)¹. This T-cell line originated from rats in which we had induced experimental autoimmune encephalomyelitis (EAE) by immunizing them against BP. Inoculation of syngeneic rats with the T-cell line led to the relatively rapid onset of EAE¹. We report here that attenuation of this cell line provides an agent for establishing resistance to induction of active EAE. Intravenous (i.v.) inoculation of syngeneic rats with cells of the line attenuated by treatment with irradiation or mitomycin C augmented resistance to EAE caused by an encephalitogenic challenge with BP. Thus, aetiological agents of autoimmune disease, like those of microbial disease, when suitably attenuated can be used as effective vaccines.

EAE can be induced in susceptible animals such as rats, guinea pigs, rabbits, monkeys² or man³ by injecting them with BP emulsified in an adjuvant such as complete Freund's adjuvant (CFA). In Lewis rats the disease is characterized by paralysis that is most marked in the tail and hind limbs and which starts usually ~12 days after a single injection of BP in CFA. Histologically the central nervous system shows perivascular infiltrates of mononuclear cells⁴. Unless the rats are aged or have undergone splenectomy or thymectomy⁵ they recover spontaneously from clinical paralysis after a number of days. To study the pathophysiology of EAE we have isolated and propagated *in vitro* a line of Lewis rat T lymphocytes that reacts only against BP, designated Z1a (ref. 1). We found that i.v. inoculation of as few as 10^3 cells of the Z1a line led to the onset of paralysis in ~4 days. Inoculation of 10^6 or more cells produced paralysis in ~2–3 days. Most rats recovered from this form of EAE if properly nursed during their paralysis. Table 1 shows the specificity of the proliferative response of the anti-BP Z1a line compared with that of the Z1c line which had been selected for its reactivity against another antigen, the purified protein derivative (PPD) of the mycobacteria present in CFA. The cells of each line responded to its specific antigen, and were also activated by the T-cell mitogen concanavalin A (Con A). Essentially all the cells in both the Z1a and Z1c lines proved to be positive for a T-cell marker using a specific monoclonal antibody (Sera-lab, UK; clone W3/13 HLK) in an immunofluorescence assay⁶.

We investigated the effect of attenuating the Z1a line by inhibiting its cell division. Table 2 shows that i.v. injection of 1×10^7 untreated cells of the Z1a line into syngeneic Lewis rats produced EAE in 18 of 20 rats within 2–3 days. Irradiation of the cells with 1,500 rad or treatment with mitomycin C, agents that block cell division, abrogated the ability of these cells to cause EAE. None of 25 rats that received Z1a cells treated in this way developed EAE. Furthermore, inoculation of

Table 1 Anti-BP and anti-PPD T-cell lines are immunospecific

T-cell line	No antigen	Proliferative response (c.p.m. $\times 10^{-3} \pm$ s.d.)		
		BP	PPD	Con
Anti-BP (Z1a)		1.7 \pm 0.3	48.7 \pm 6.1	1.9 \pm 0.4
Anti-PPD (Z1c)		1.4 \pm 0.7	1.2 \pm 0.4	77.9 \pm 10.4

The Z1a and Z1c cell lines originated from the same draining lymph node cell population obtained from female Lewis rats immunized with BP in CFA as described elsewhere¹. To develop the cell lines, Lewis were injected in each footpad with 100 µl 50% containing BP (25 µg/ml) extracted from guinea pig spinal cords¹⁰ emulsified in equal volume phosphate-buffered saline and CFA containing 4 mg/ml¹¹ of *Mycobacterium tuberculosis* H₃₇Rv (Difco). On day 9, the draining lymph nodes were removed and a single-cell suspension prepared. The cells were selected *in vitro* for BP or PPD by culturing them with either antigen for 72 h. The lymphoblasts that were generated were separated by a continuous Ficoll gradient and propagated and maintained *in vitro* cell line for several months in medium enriched with T-cell growth factors as reported elsewhere¹. The proliferative responses of the T-cell lines were tested *in vitro* as follows¹. Briefly, 2.5×10^4 cells of either Z1a or Z1c lines were cultured in quadruplicate in flat-bottom microtitre wells in 0.2 ml of Eagle's medium supplemented with 1% fresh autologous serum, 2-mercaptoethanol (5 $\times 10^{-3}$ M), L-glutamine (2 $\times 10^{-3}$ M) and antibiotics (streptomycin and penicillin) with added irradiated (1,500 rad) normal syngeneic lymph node cells as accessory cells (5 $\times 10^3$ cells ml⁻¹) and antigens, BP (50 µg ml⁻¹), or PPD (25 µg ml⁻¹; Stat Serum Institut) or Con A (2.5 µg ml⁻¹; Miles-Yeda, Israel). After 24 h, the cultures were pulsed with ³H-thymidine (1 µCi per well, specific activity 10 Ci mmol⁻¹; Nuclear Research Centre, Israel) for 16 h. Cells were then collected on glass fibres using an automatic collector, and thymidine incorporation measured in a liquid scintillation counter.

untreated cells of the Z1c line also failed to induce EAE. The induction of EAE is a function of the specific anti-BP Z1a line, a property lost after irradiation or treatment with mitomycin C.

We then tested whether inoculation with cells incapable of inducing EAE could affect the susceptibility of rats to active induction of EAE by later challenge with BP in CFA. Table 2 shows that untreated Lewis rats were highly susceptible to induction of EAE on injection with BP in CFA; 69 of 71 rats developed disease. Intravenous inoculation of cells of the Z1c anti-PPD line, either untreated or irradiated, did not affect the susceptibility and EAE was induced in all 20 rats challenged with BP in CFA. In contrast, a single i.v. injection of 1×10^7 Z1a cells attenuated by treatment with mitomycin C or irradiated led to significantly increased resistance to induction of EAE. Only 14 of a total of 40 rats showed any signs of paralysis and the degree of the paralysis in these rats was judged to be milder than that appearing in the other groups. Thus it seems that vaccination with attenuated autoimmune T lymphocytes

Table 2 Attenuated T lymphocytes of the Z1a anti-BP line do not produce EAE

Line	Treatment	Inoculation of T-cell lines
Anti-BP (Z1a)	Untreated	18/20
	Irradiated	0/15
	Mitomycin C	0/10
Anti-PPD (Z1c)	Untreated	0/20

Healthy female Lewis rats (2–3 months old) were injected i.v. with 1×10^7 cells of T-lymphoblast cell lines specifically reactive against Z1a or Z1c (ref. 1). Before inoculating the cell lines into normal syngeneic animals, they were re-stimulated *in vitro* with the relevant antigen, in the presence of irradiated (1,500 rad) syngeneic accessory cells for 72 h (ref. 1). The cells, >80% lymphoblasts, were then collected and injected, either untreated or attenuated by irradiation (1,500 rad from a ⁶⁰Co source, or treatment with mitomycin C (50 µg per 10⁶ cells ml⁻¹; Sigma) at 37°C for 40 min. The treated cells were washed extensively before being inoculated. EAE was diagnosed clinically as overt paralysis of the hind limbs and histologically by perivascular mononuclear cell infiltration of the central nervous system.

Incidence of EAE in response to injection of BP	% Inhibition of EAE
69/71	—
10/10	0
10/10	0
8/25*	68
6/15*	60

induced in naive Lewis rats (2-3 months old) or in animals immunized i.v. 3 weeks earlier with 10^7 cells of the B1-1 T-cell line or with cells of the anti-BP Z1a T-cell line that irradiated (1,500 rad) or treated with mitomycin C, as in Table 2 legend. EAE was induced by injecting BP in CFA intradermally in the ear pads of the animals, as described in Table 1 legend.

injection against active EAE for about 65% of the

know the mechanism by which the attenuated T cells confer increased resistance to induction of EAE; it seems reasonable to suspect that some process of cell-cell communication is involved. The Z1a anti-BP lymphocytes probably act on the ineffective Z1a anti-PPD lymphocytes in their antigen receptors (Table 1). Antigen receptors can bind to antigens as well as B lymphocytes or antibodies¹ can bind to antigen. Immunity against antigen receptors, anti-receptor immunity, has been proposed to serve as a mechanism to regulate immune responses by suppressing or activating the activity of lymphocytes bearing the target receptors¹. Lymphocytes with attenuated Z1a cells might have produced an immune response against endogenous clones of lymphocytes with BP receptors. As anti-BP clones are the aetiological agent in the development of the disease would be inhibited. Our results could be explained by anti-receptor antibodies against the autoimmune lymphocytes that are induced by the antigen. However, other explanations are possible and this hypothesis must be tested experimentally. In any case, the mechanism of protection, the procedure here can be conceptually related to vaccination against diseases in which inoculation of an attenuated microorganism induces a degree of protection against the antigen. In the case of autoimmunity, the aetiological agent is not a microbe, but arises within the immune system of the individual. Our results indicate that an artificially induced immune disease may be mitigated or prevented by an immune response against specific effector lymphocytes. A different problem is posed by the need to treat the spontaneous chronic processes that characterize the important immune diseases of man.

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C-terminal sequence of the secreted form of mouse IgD heavy chain

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Immunoglobulins have been identified as membrane-bound molecules on the surface of B lymphocytes and as secreted products of plasma cells. In the case of immunoglobulin M (IgM) the carboxy-terminal sequences of the μ -chain of membrane-bound and secreted molecules differ from each other and are encoded by different exons of the μ constant region ($C\mu$) gene. The coding sequence for the C-terminus of the secreted μ -chain is contiguous with the 3' end of the $C\mu 4$ exon and separate exons downstream of $C\mu 4$ encode the C-terminus of the membrane-bound chain¹⁻³. Immunoglobulin D is also found membrane-bound and as a secreted molecule, and recent data indicate that the exon arrangement of the $C\delta$ gene is in part similar to that of the $C\mu$ gene⁴⁻⁶. However, the amino acid sequence analysis presented here demonstrates that in the case of IgD the C-terminus of the secreted δ -chain is encoded by a separate exon (the $C\delta DC$ exon of Tucker *et al.*⁷) and not by the $C\delta AC$ sequence which corresponds topographically to the sequence expressed at the C-terminus of secreted μ chains.

The cell line B1-8.61 (IgD, $\lambda 1$) has been isolated as a switch variant of the cell line B1-8.64.1 (IgM, $\lambda 1$) and is of C57BL/6 origin⁸. It secretes a monoclonal IgD antibody with specificities for the hapten (4-hydroxy-3-nitrophenylacetyl) (NP). Anti-NP antibodies from B1-8.61 ascites fluid were purified by affinity chromatography⁹. The heavy chains of these molecules are linked to each other and to the light chains by disulphide bridges (ref. 6 and unpublished data). After complete reduction and carboxyamidomethylation the heavy and light chains were eluted from Sephadex G-100 with 5.6 M urea, 1 M propionic acid

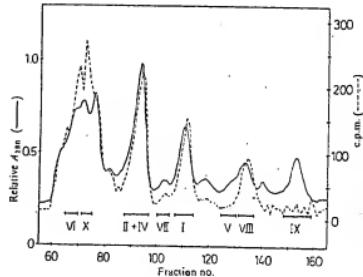


Fig. 1 Gel filtration of cyanogen bromide-cleaved peptides of ^{14}C -carboxyamidomethylated B1-8.61 heavy chain. Cyanogen bromide cleavage of 50 mg of the completely reduced and alkylated⁹ heavy chain was performed in 70% formic acid at a concentration of 10 mg ml^{-1} with a fivefold excess (w/w) of CNBr for 24 h at 20 °C. The mixture was dried under a stream of nitrogen. The peptides were dissolved in 3.5 ml of 0.1 M formic acid containing 6 M deionized urea and applied to a Sephadex G-50 superfine column (2 \times 200 cm) equilibrated in the same solvent. Fractions of 3.5 ml were collected and aliquots of 20 μl were used for liquid scintillation counting in 5 ml of Bray's solution¹⁰. Roman numerals of the pooled fractions refer to the position of the corresponding peptides in the sequence of the B1-8.61 heavy chain as given in Fig. 2. Peptide no. III was insoluble in 6 M urea, 0.1 M formic acid, and could thus be isolated without chromatographic separation.